DECLARATION

I, Nakayuki YAMAMOTO, the undersigned, state that my residence address is 55-1, Aza-Nokubi, Hichiya, Hyuga-shi, Miyazaki-ken, 833-0062/Japan, and that my background of technical qualification is as follows:

I had graduated Master Course of Pharmacology, Meijo University, Japan in March 1972. I was employed by Toyo Jozo Kabushiki Kaisha (new Asahi Kasei Kogyo K. K.). During the employment, my major works were studies on injectable formulation of pharmaceutical products, developmental studies on new DDS (drug delivery systems)(e.g. percutaneous absorption preparations, oral preparations, rectal absorption preparations, nasal absorption preparations, etc.), and nasal absorption preparation of elcatonin. All of my major study works were research and development of formulations of pharmaceuticals. Since February 1996, I was appointed a manager of quality control section, a chief quality control manager, in Hyuga Pharmaceutical Plant, Asahi Kasei Kogyo K. K.

I am an author or coauthor of the scientific papers and I am am inventor or coinventor of the patents and patent applications, several of which are listed hereinbelow.

U.S. Patent No. 5,281,580

U.S. Patent No. 5,407,911

Japanese Patent Application No. 4-147121

Japanese Patent Application No. 63-144704

Japanese Patent Application No. 63-256919

Japanese Patent Application No. 63-288886

Nasal absorption of elcatonin in rats

- report in Japan-U.S. Joint Pharmacology Congress, December 1987.

Following experimental works were performed by myself with the cooperation of my coleagues.

1. Object of the experiments

An object of the experiments is to confirm an effect of the present invention, i.e. an effect of bioactive peptide on mucosal absorption, by comparison with three tests; use of known absorption promotor by known technique; use of a compound having vasodilating action; and use of absorption promotor and vasodilator.

2. Experimental

Materials and methods:

Three types of preparations containing bioactive peptide, i.e. elcatonin, listed in the following are prepared and are administered intranasally in rats. Plasma levels of elcatonin after administration are measured by way of time dependent manner.

(a) elecatonin 400 units (bioactive peptide)
sodium glycocholate 5 mg (absorption promotor)
in 1 ml phosphate buffer
(referential example 1)

(b) elecatonin 400 units (bioactive peptide)
diltiazem hydrochloride 5 mg (vasodilator)
in 1 ml phosphate buffer
(referential example 2)

(c) elecatonin 400 units (bioactive peptide)

sodium glycocholate 5 mg (absorption promotor)
diltiazem hydrochloride 5 mg (vasodilator)
in 1 ml phosphate buffer
(example 1)

[Preparation of the formulation for experiments]

(a) Sodium glycocholate (SGC: Sigma Inc., U.S.A.) 5mg was dissolved in isotonic phosphate buffer pH 6.0 1ml. The isotonic solution 1 ml was added to elcatonin 400 units/vial (lyophilized) and dissolved to prepare elecatonin 400 units solution in phosphate buffer 1 ml.

(b) Diltiazem hydrochloride (DTZ: Sigma Inc., U.S.A.) 10 mg was dissolved in isotonic phosphate buffer pH 6.0 2 ml. The solution 1 ml was added to elcatonin 400 units/viral (lyophilyzed) to prepare elcatonin 400 units solution in phosphate buffer 1 ml.

(c) Diltiazem hydrochloride (DTZ: Sigma Inc., U.S.A.) 10 mg was dissolved in 0.5 % sodium glycocholate solution (pH 6.0 isotonic phosphate buffer) 2 ml to prepare solution containing 0.5 % and 0.5 % DTZ. This solution 1 ml was added to elcatonin 400 units/vial (lyophilized) to prepare elcatonin solution 400 units in 1 ml of phosphate buffer solution of SGC and DTZ.

[in vivo absorption tests in rats]

Wister rats, male, 200-250 gr. SLC Japan, which were fasted for overnight, 4 rats in a group, were anesthetized with pentobarbital (nembtal inj., Dainippon Seiyaku K.K., Japan). Rats were

tracheotomized and were intratracheally incubated with polyethylene tube. Subsequently rats were esophagotomized and the tube was inserted into the postnasal space. The drug preparation before using, 25 μl, was administration into the right nasal cavity by using micropipet. Blood 200μl was collected through the femoral vein by time dependent manner before and after administration. The collected blood was centrifuged (15,000 rpm for 10 minutes at 5 ℃). The thus obtained plasma was stored at - 30 ℃ before use for analysis.

Plasma elcatonin level was measured by RIA.

[Results]

Time course changes the plasma elcatonin lelvels were shown in the figure.

Significant increase in plasma elcatonin levels was observed in the group administered with the preparation (c) elcatonin with addition of 0.5 % sodium glycocholate and 0.5 % diltiazem hydrochloride (-▲-) as compared with the group administered with the preparation (a) elcatonin with addition of 0.5 % sodium glycocholate (-■-). An area under the blood concentration-time curve (AUC) of the preparation (c) administered group was about 3.6 times large than AUC of the preparation (a) administered group, the result of which showed significant increase in absorption of elcatonin through nasal mucosa.

The preparation (b) elecatonin with addition of 0.5 % diltiazem (-●-) showed almost no absorption through nasal mucosa.

[Discussion]

Sodium glycocholate is an absorption promotor which promotes absorptiopn of bioactive peptide through mucosal membrane.

Diltiazem hydrochloride is a calcium channel inhibitor having vasodilating activity.

The present experiments clearly showed that significant high absorption of bioactive peptide through mucosa was provided by using the preparation (c) in which the absorption promotor and vasodilator were combining used as compared with using the preparation (a) in which the absorption promotor was used. The use of vasodilator (b) was shown to have no absorption promoting activity for bioactive peptide. Namely diltiazem hydrochloride per se has no absorption promoting activity.

Good absorption of bioactive peptide through mucosa was shown to be achieved by using the preparation combined with absorption promotor and vasodilator as compound with using the preparation with absorption promotor. Absorption of bioactive peptide could not be achieved through mucosa by using a compound having vasodilating activity.

And I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willfull false statements and the like so made are punishable by fine or imprisonment, or both, under 1001 of Title 18 of the United States Code and that

such willfull false statements may Jeopardize the validity of the application or any patent issuing thereon.

Dated this 2nd day of October, 1998

Nakayuki Yamamoto

Nakayuki YAMAMOTO